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# Improving microcirculation with therapeutic intrathoracic pressure regulation in a porcine model of hemorrhage

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ABSTRACT

*Aim of study:* Intrathoracic pressure regulation (IPR) has been used to treat hypotension and states of hypoperfusion by providing positive pressure ventilation during inspiration followed by augmentation of negative intrathoracic pressure during expiration. This therapy augments cardiac output and lowers intracranial pressure, thereby providing greater circulation to the heart and brain. The effects of IPR on microcirculation remain unknown.

*Methods:* Using a hemorrhagic model, hemodynamics and sublingual microcirculation were evaluated after a 55% blood loss over a 30 min timeframe in 10 female farm pigs (30 kg) previously anesthetized with isoflurane.

*Results:* After hemorrhage the mean arterial pressure was 27 ± 4 mm Hg. Blood cell velocity, the key indicator of microcirculation, was significantly reduced after the bleed from 1033 ± 175 μm/s pre-bleed to 147 ± 60 μm/s ($p < 0.0001$). Application of an IPR device reduced airway pressure during expiration to −9 mm Hg after each positive pressure breath (10 mL/kg, 10 breaths/min) and resulted in a rapid increase in systemic hemodynamics and microcirculation. During IPR treatment, average mean arterial pressure increased by 59% to 43 ± 6 mm Hg ($p = 0.002$) and blood cell velocity increased by 344% to 506 ± 99 μm/s ($p = 0.001$).

*Conclusion:* In this animal model, we observed that microcirculation and systemic blood pressures are correlated and may be significantly improved by using IPR therapy.



## 1. Introduction

During hemorrhagic shock, macrohemodynamic variables such as central blood pressure (BP), heart rate and cardiac output are generally used to determine shock severity and aid in determining treatment measures. These parameters are characteristically altered due to insufficient circulating blood volume thus yielding inadequate organ perfusion.[1] There exists increasing literature evidence demonstrating that these global parameters may not be reflective of changes in microcirculation such as capillary perfusion.[2–5] Furthermore, following hemorrhagic shock management, notable variations in microcirculation may still persist and could contribute to organ failure. A better therapeutic guide might include information regarding microhemodynamics.

Flow through the microcirculatory network of small vessels, including arterioles, capillaries and venules is regulated through interactions between these vessels according to local and global demand. Sidestream dark-field microscopy (SDM) is a novel and noninvasive technique for direct *in vivo* visualization and quantification of microcirculation.[6,7] The technique of SDM can assess blood flow in microvessels of ≤20 μm and has been validated in animal models of hemorrhagic shock.[8–13] These studies have demonstrated that SDM images are comparable or superior to those achieved with either intravital fluorescence videomicroscopy or capillaroscopy.[14,15]

Recently studies in animals and humans have demonstrated that a decrease in intrathoracic pressure during the expiratory phase of positive pressure ventilation enhanced venous blood flow to the heart under euvolemic and hypotensive conditions.[16–21] Intrathoracic pressure regulation (IPR) has been developed and used to treat animals in severe hypovolemic and hypotensive states by maintaining a constant negative intrathoracic pressure except

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during intermittent positive pressure ventilation.[22] The decrease in intrathoracic pressure enhances venous blood flow to the heart in circumstances of low blood pressure.[16–20] The IPR improves mean arterial, cerebral and coronary perfusion pressures while decreasing right atrial and intracranial pressures. Until the present study, however, the effect of IPR on microhemodynamics had not yet been assessed. Utilizing SDM to assess microcirculation in the setting of profound hemorrhagic hypotension, the objectives of the present study were (1) to demonstrate the relationship between macro- and microhemodynamics and (2) to assess the effect of IPR on microcirculatory flow. We tested the hypothesis that continuous, controlled negative intrathoracic pressure regulation after each positive pressure breath, will increase both microcirculation and macrocirculation simultaneously in a severe hypovolemic anesthetized animal model.

## 2. Methods

### 2.1. Experimental preparation

All animal studies were approved by the Institutional Animal Care Committee of the Minneapolis Medical Research Foundation at Hennepin County Medical Center. All animals received treatment and care in compliance with the 1996 Guide for the Care and Use of Laboratory Animals by the National Research Council in accordance with the USDA Animal Welfare Act, PHS Policy, and the American Association for Accreditation of Laboratory Animal Care.

Ten female farm pigs (30 kg, domestic crossbreed) were fasted overnight. They were sedated with 10 mL (100 mg/mL) of intramuscular ketamine HCl (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA, USA). The animals were intubated with a 7.5 mm cuffed French endotracheal tube inflated to prevent air leaks and anesthetized with isoflurane and remained anesthetized throughout the study with doses ranging from 0.5% to 1.5%. Positive pressure, volume control ventilation with a tidal volume of 10 mL/kg and $O_2$ was delivered with a NarkoMed 4A (North American Drager, Telford, PA, USA) ventilator. The respiratory rate was adjusted (average 12 $\pm$ 2 bpm) to keep oxygen saturation above 96% and end-tidal $CO_2$ between 38 and 42 mm Hg.

The surgical preparation phase had been described previously.[23] Briefly, while in ventral recumbency, an intracranial bolt was inserted into the animal's parietal lobe to measure intracranial pressure using a 3.5-French micromanometer pressure transducer (Miko-Tip Transducer, Millar Instruments, Inc., Houston, TX, USA). Animals were then placed supine. The left femoral artery and left external jugular vein were cannulated using a modified Seldinger percutaneous technique. Central aortic blood pressures were measured continuously via a micromanometer-tipped Millar catheter placed in the chest cavity at the level of origin of the thoracic descending aorta. Central venous blood pressures were measured via a micromanometer-tipped Millar catheter placed in the superior vena cava, approximately 2 cm above the right atrium. Carotid artery blood flows were measured using a bidirectional Doppler flow probe attached to the internal carotid artery (Transonic Systems, Ithaca, NY, USA). Surface ECG was also monitored continuously. A thermometer was placed in the rectum and core body temperature maintained with a heating blanket between 37.0°C and 38.0°C throughout the study. All data were digitized using a computer data analysis program (BIOPAC MP 150, BIOPAC Systems Inc., Goleta, CA, USA). $EtCO_2$, respiratory rate, and arterial oxygen saturation were recorded with a $CO_2$SMO Plus (Novametrix Medical Systems, Wallingford, CT, USA).

### 2.2. Experimental protocol

Prior to beginning the experimental protocol, succinylcholine (93 μg/kg/min) (Hospira, Lake Forest, Il, USA) was administered to all animals to inhibit the spontaneous gasping reflex that can be associated with use of an IPR device (CirQLATOR™, Advanced Circulatory Systems Inc., Roseville, MN, USA). The IPR device, shown in Fig. 1, was attached to the anesthesia machine, and was used to rapidly lower airway pressures to −9 mm Hg after each positive pressure ventilation (PPV). Following a 10 min stabilization period, baseline hemodynamic parameters, blood gas and microcirculatory measurements were recorded. Animals were bled to 50% of their blood volume and the bleed was interrupted if the animal's systolic aortic pressure fell below 30 mm Hg. Blood volume, removed over 30 min with a peristaltic pump to simulate a venous bleed, was estimated using the formula: total blood volume = weight of animal (kg) × 65 mL/kg. Upon completion of the hemorrhage, animals were given a 10 min stabilization period after which a hemorrhagic baseline (HBL1) was recorded over a period of 3 min. If microcirculatory flow did not appear to be grossly altered after the 50% bleed, a second smaller bleed was performed in order

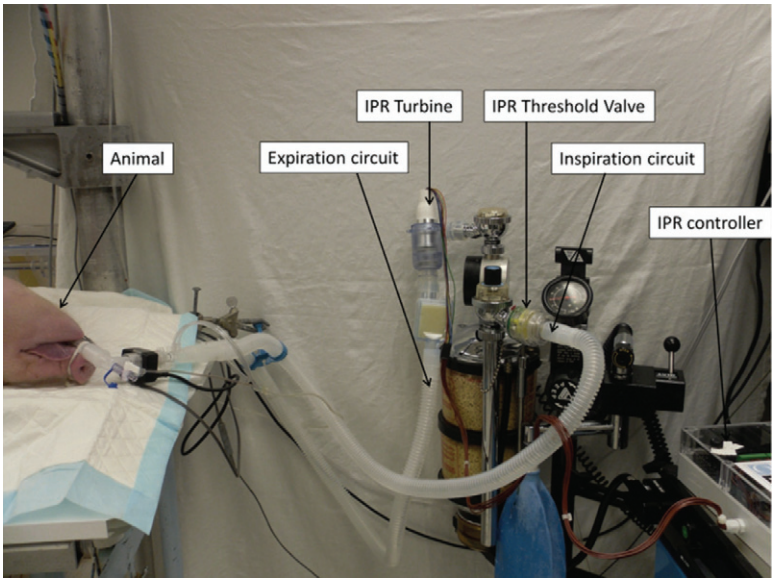


**Fig. 1.** Photo demonstrating use of the IPR in the ventilation circuit. The IPR turbine generates the level of intrathoracic pressure determined by the operator using both the IPR Controller and an IPR impedance threshold valve.

to obtain an aortic systolic pressure between 35 and 45 mm Hg. The maximum hemorrhage volume was 55%. Following a second 10 min stabilization period, a second hemorrhagic baseline (HBL2) was recorded over a period of 3 min. The data obtained at HBL2 were used as the study hemorrhagic baseline (HBL), if no secondary bleed was needed then HBL1 was used as study HBL for all data analyses. At this point, animals were randomized to one of two study groups (5 animals per group): A) 30 min IPR followed by 30 min of PPV or conversely, B) 30 min PPV followed by 30 min IPR and then 30 min PPV. During IPR, the ventilation rate was 10 breaths per min, the tidal volume 13 mL/kg, and the $FiO_2$ was adjusted to maintain $SpO_2$ >95%. During the PPV intervention, the ventilation rate was 10 breaths per min, the tidal volume 10 mL/kg, and the $FiO_2$ was adjusted to maintain $SpO_2$ >95%. At the end of the second PPV phase or if blood pressure decreased to <30 mm Hg, 500 mL of blood was transfused over 10 min and an assessment of hemodynamic and microcirculation parameters was conducted. If the animals in study group B exhibited a further decrease in blood pressure to <30 mm Hg during the first PPV treatment phase, IPR was rapidly initiated to prevent the pigs from dying midway through the study. Hemodynamic and microcirculatory data were measured at intervals 5, 15 and 30 min during each intervention and after completion of the blood transfusion. At the end of the study, the isoflurane concentration was increased to 5% and the animal was euthanized with a bolus i.v. injection of 10 M KCl (30 mg/kg).

### 2.3. Data analysis

Lingual mucosal microcirculation was assessed via SDM using a MicroScan imaging device (MicroVision Medical, Amsterdam, Netherlands). The 5× optical probe, encompassing a 1025 μm × 750 μm field, was applied manually under the animal's saline-moistened tongue. Five distinct fields were documented at each aforementioned time interval and digitally saved via a computer recording system. The microcirculatory data were recorded at a rate of 30 frames/s. Arteriolar density, blood flow velocity and score velocity were calculated via a frame by frame analysis by two individuals blinded to the intervention as described previously by others.[2,24] Briefly, arteriolar density was determined by counting the number of vessels intersecting a set of grid lines of known length. Arteriolar blood flow analysis was conducted by assessing four arterioles per field per animal per time period and results are depicted as a mean for each intervention. Blood flow velocity was calculated by measuring the rate at which red blood cells travel a 200 μm distance through individual arterioles (<20 μm) and mean score velocity was assessed via a standard classification scale. The velocity classification scale was as follows: 0 = no flow, 1 = sludging, 2 = moderate flow and 3 = normal flow.[24] Arterial blood gas and hemodynamic parameters were evaluated at the same time intervals that microcirculation was assessed; during baseline, after the bleed, during minutes 5, 15 and 30 of the interventions and after the blood transfusion. Mean arterial pressure (MAP) was calculated as the sum of the aortic systolic pressure and twice the aortic diastolic pressure, divided by three. Cerebral perfusion pressure was calculated as the difference between aortic mean pressure and mean intracranial pressure. Carotid blood flow was calculated by numerically integrating values for the anterograde minus the retrograde flow recorded over 1 min.

The primary end point was the change in microcirculation as determined by arteriolar blood flow velocity. All values with a normal distribution are expressed as Mean ± SEM. Results were compared using a paired Student's *t* test. *P* values of <0.05 were considered statistically significant. Statistical analyses were performed with SPSS® Statistics 17.0 (IBM Corporation, Somers, NY, USA).

## 3. Results

A 50% hemorrhage was inadequate to achieve a significant reduction in microcirculation in 6/10 animals and they were thus subjected to additional blood loss of up to 55% as described in the Methods. Only 2/10 animals were able to sustain at least 15 min of the second PPV control phase with a systolic blood pressure >30 mm Hg. Blood was transfused in only 4 animals; the others were too sick to survive at that point or had died in earlier interventions. All animals randomized to Group A survived the 30 min of IPR and 4/5 died during the post-IPR 30 min control period. The one surviving pig was able to complete the 30 min post-IPR intervention and received the blood transfusion. In the animals randomized to Group B, three pigs survived the first 30 min control intervention and two developed further hypotension: they were treated with IPR prior to the completion of the 30 min control intervention earlier than anticipated to prevent death. All animals in Group B were alive after the IPR treatment and one pig completed the 30 min of the second non-IPR intervention and 3 received a blood transfusion before the 30 min interval to prevent further hypotension and maintain viability.

The hemodynamic and blood gas parameters associated with this acute model of hemorrhage are shown in Table 1. Severe hypotension was associated with a significant reduction in aortic pressure, MAP, cerebral perfusion pressure, mean right atrial pressure and mean carotid blood flow along with an increase in heart rate. A significant reduction in hematocrit levels was also observed at hemorrhagic baseline when compared with the study baseline. Prior to the bleed MAP was 59 ± 4 mm Hg and it decreased with the hemorrhage to 27 ± 4 mm Hg ($p$ = 0.002) (Fig. 2). For pigs randomized to Group B (no IPR initially) MAP remained low. By contrast, whether IPR was randomized first or second, there was a rapid rise in MAP by 59% to an average value of 43 ± 6 mm Hg ($p$ = 0.002 compared with values prior to IPR). When IPR was removed MAP decreased rapidly to a mean of 27 ± 9 mm Hg ($p$ = 0.033 as compared values with IPR). Following the blood transfusion, MAP increased to 54 ± 10 mm Hg.

The changes in microcirculation, as determined by the mean arteriolar blood flow velocity, were the primary focus of this study. The results are shown in Table 2 and Fig. 3. Under baseline pre-bleed conditions, the mean arteriolar blood flow velocity was 1033 ± 175 μm/s and this decreased by 86% to 147 ± 60 μm/s after the bleed ($p < 0.001$). Use of IPR rapidly improved the mean microcirculation blood flow and mean score velocities in comparison to the values obtained after hemorrhage ($p < 0.001$). Within 5 min of IPR, blood flow improved by 344% to 506 ± 99 μm/s and the mean score velocity increased to 2.25 ± 0.03 μm/s in comparison to post-bleed values before IPR of 147 ± 60 μm/s and 0.97 ± 0.04 μm/s, respectively. Blood flow velocity after 15 and 30 min of IPR therapy was 544 ± 123 μm/s and 526 ± 140 μm/s (Table 1 and Fig. 1). Mean score velocity was 2.25 ± 0.03 μm/s and 2.48 ± 0.02 μm/s at 15 and 30 min (Table 1 and Fig. 2). An immediate decline in blood flow and mean score velocities occured when IPR was stopped. After 5 min without IPR, blood flow declined to 61 ± 67 μm/s and the mean score velocity decreased to 0.49 ± 0.04 μm/s ($p < 0.0001$). Four animals survived long enough to be treated with a 500 mL autologous blood transfusion. The transfusions resulted in a rapid and significant increase in arteriolar microcirculation flow to 569 ± 126 μm/s, approximately the same as that achieved with IPR alone and half of the initial euvolemic value. The mean arteriolar density was constant throughout the interventions. In addition, there were no untoward consequences or safety issues observed with IPR application.

Blood gas values, as shown in Table 1, demonstrate that the pigs developed severe acidosis, as reflected in the base excess values of −8.1 after the initial bleed in the absence of IPR. The base excess

**Table 1**
Mean ± SEM values of the hemodynamic and arterial blood gas parameters during each intervention.

| | Study baseline | HBL1 | HBL2 | HBL | Pre-IPR 5 min (Control) | Pre-IPR 15 min (Control) | Pre-IPR 30 min (Control) | IPR 5 min | IPR 15 min | IPR 30 min | Post-IPR 5 min (Control) | Blood transfusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hemodynamic parameters | | | | | | | | | | | | |
| Ao sys (mm Hg) | 75 ± 5‡ | 51 ± 6 | 30 ± 4 | 37 ± 5 | 39 ± 7 | 49 ± 12 | 41 ± 11 | 61 ± 7‡ | 58 ± 7‡ | 51 ± 9* | 34 ± 8 | 75 ± 8‡ |
| Ao dia (mm Hg) | 50 ± 4‡ | 31 ± 4 | 17 ± 3 | 23 ± 3 | 23 ± 6 | 28 ± 8 | 24 ±11 | 34 ± 5‡ | 34 ± 5‡ | 27 ± 7* | 21 ± 7 | 44 ± 11* |
| MAP (mm Hg) | 59 ± 4‡ | 38 ± 4 | 19 ± 2 | 27 ± 4 | 29 ± 7 | 29 ± 11 | 30 ± 11 | 43 ± 6‡ | 42 ± 6‡ | 36 ± 7* | 27 ± 9 | 54 ± 10‡ |
| CePP (mm Hg) | 44 ± 4‡ | 20 ± 4 | −2 ± 6 | 10 ± 6 | 24 ± 7* | 25 ± 9 | 21 ± 12 | 38 ± 6‡ | 34 ± 8‡ | 25 ± 7 | 9 ± 12 | 45 ± 13‡ |
| RA (mm Hg) | 5.0 ± 1.5‡ | 0.8 ± 0.9 | 1.7 ± 0.9 | 1.3 ± 0.9 | 1.8 ± 0.5 | 2.1 ± 0.3 | 2.2 ± 0.22 | −4.5 ± 2.0 | −7.0 ± 1.4‡ | −7.0 ± 1.9‡ | 0.6 ± 3.7 | −0.5 ± 5.4 |
| ITP (mm Hg) | 17 ± 12 | 4 ± 2 | 6 ± 0.4 | 4 ± 2 | 7 ± 0.4 | 7 ± 1 | 6 ± 1 | −8 ± 1‡ | −9 ± 1‡ | −10 ± 1‡ | 7 ± 0.3 | 3 ± 4 |
| ICP (mm Hg) | 18 ± 5 | 20 ± 5 | 23 ± 7 | 19 ± 4 | 7 ± 0.2 | 13 ± 3 | 13 ± 3 | 8 ± 5 | 5 ± 3* | 5 ± 2‡ | 14 ± 2 | 14 ± 3 |
| CBF (mL/min) | 111 ± 29 | 80 ± 23 | 41 ± 17 | 62 ± 15 | 67 ± 20 | 62 ± 21 | 50 ± 23 | 119 ± 18‡ | 114 ± 19‡ | 116 ± 31 | 60 ± 25 | 131 ± 23* |
| HR (bpm) | 103 ± 7‡ | 201 ± 11 | 209 ± 10 | 199 ± 11* | 206 ± 1 | 208 ± 3 | 220 ± 4 | 195 ± 8 | 199 ±11 | 187 ± 9 | 203 ± 10 | 184 ± 5 |
| RR | 13 ± 0.9 | 14 ± 0.8 | 12 ± 0.6 | 14 ± 0.8 | 15 ± 1.1 | 15 ± 1.1 | 15 ± 1.1 | 14 ± 0.7 | 13 ±0.8 | 14 ±0.9 | 13 ± 0.7 | 12 ± 2.2 |
| Number of animals | 10 | 10 | 4 | 10 | 4 | 3 | 3 | 10 | 10 | 10 | 5 | 4 |
| Blood gas parameters | | | | | | | | | | | | |
| Arterial pH | 7.5 ± 0‡ | 7.4 ± 0 | 7.4 ± 0 | 7.4 ± 0 | 7.3 ± 0.1 | 7.3 ± 0.1 | 7.3 ± 0.1 | 7.2 ± 0.1‡ | 7.2 ± 0.1† | 7.2 ± 0.1† | 7.2 ± 0.10 | 7.3 ± 0.1 |
| $PaCO_2$ | 35 ± 1† | 30 ± 2 | 23 ± 2 | 25 ± 2 | 27 ± 1 | 26 ± 2 | 27 ± 4 | 31 ± 4 | 31 ± 4 | 34 ± 6 | 39 ± 10 | 42 ± 1 |
| $PaO_2$ | 163 ± 20 | 151 ± 20 | 176 ± 36 | 123 ± 12 | 157 ± 26 | 166 ± 27 | 156 ± 24 | 117 ± 24 | 116 ± 32 | 115 ± 31 | 108 ± 16 | 104 ± 19 |
| Hct | 26.6 ± 0.7‡ | 23.4 ± 0.2 | 21.0 ± 1.0 | 21.8 ± 0.7 | 22.3 ± 0.3 | 22.7 ± 1.2 | 25.3 ± 1.7 | 23.4 ± 1.3 | 23.5 ± 1.6 | 23.5 ± 1.9 | 26.3 3.5 | 30.0 ± 3.0 |
| Arterial $HCO_3$ | 26.0 ± 0.6‡ | 19.8 ± 0.9 | 14.7 ± 1.1 | 16.3 ± 1.4 | 15.2 ± 2.9 | 14.7 ± 3.0 | 14.8 ± 4.2 | 12.2 ± 2.7 | 12.6 ± 3.1 | 13.0 ± 3.8 | 15.8 ± 4.6 | 20.4 ± 4.4 |
| Arterial base excess | 2.4 ± 0.5‡ | −4.7 ± 0.8 | −9.8 ± 1.1 | −8.1 ± 1.4 | −10.5 ± 4.2 | −11.1 ± 4.2 | −11.4 ± 5.4 | −16.2 ± 3.4† | −15.6 ± 4.0 | −15.8 ± 4.6 | −12.1 ± 5.7 | −6.2 ± 6.0 |
| % Saturation $O_2$ | 99 ± 0 | 99 ± 0 | 99 ± 1 | 99 ± 0 | 99 ± 0 | 100 ± 0 | 99 ± 0 | 96 ± 2 | 96 ± 2 | 96 ± 3 | 95 ± 4 | 97 ± 3 |

Ao sys: aortic systolic pressure; Ao dia: aortic diastolic pressure; MAP: mean arterial pressure; CePP: mean cerebral perfusion pressure; RA: mean right atrial pressure; ITP: mean intrathoracic pressure; ICP: mean intracranial pressure; CBF: mean carotid blood flow; HR: heart rate; RR: respiratory rate; $EtCO_2$: end-tidal $CO_2$; *n*: number of animals. BL: baseline; HBL1: hemorrhagic baseline 1; HBL2: hemorrhagic baseline 2; HBL: hemorrhagic baseline; Pre-IPR 5, 15, 30 min (Control): 1st control at min 5, 15 and 30, respectively; IPR 5 min, IPR 15 min, IPR 5 min: intrathoracic pressure regulation at min 5, 15 and 30, respectively; 2nd control at min 5: Post-IPR 5 min (Control). Pressures and $EtCO_2$ in mm Hg, flow in mL/min.
*$p < 0.05$ for HBL vs. intervention; †$p < 0.03$ for HBL vs intervention; ‡$p < 0.01$ for HBL vs intervention.

**Table 2**
Mean ± SEM values of the microcirculatory parameters of arteriolar blood flow velocity, arteriolar density and mean score velocity, including the percentage of each type of vessel according their velocity score[24] (0 = no flow, 1 = sludging, 2 = moderate flow and 3 = normal flow).

| | Study baseline | HBL1 | HBL2 | HBL | Pre-IPR 5 min (Control) | Pre-IPR 15 min (Control) | Pre-IPR 30 min (Control) | IPR 5 min | IPR 15 min | IPR 30 min | Post-IPR 5 min (Control) | Blood transfusion |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Microcirculatory parameters | | | | | | | | | | | | |
| Arteriolar blood flow velocity (μm/s) | 1033 ± 175 | 304 ± 112¶¶ | 155 ± 70¶¶ | 147 ± 60¶ | 89 ± 70 | 80 ± 58 | 76 ± 77 | 506 ± 99* | 544 ± 123** | 526 ± 140*** | 61 ± 67 | 569 ± 126 † |
| Arteriolar density (vessel/$\mu m^2$) | 26.6 ± 0.9 | 25.6 ± 0.9 | 24.1 ± 1.4 | 23.1 ± 0.9 | 21.0 ± 1.4 | 21.2 ± 1.3 | 21.9 ± 1.2 | 26.8 ± 0.9§ | 29.5 ± 1.0§§ | 26.5 ± 1.9 | 28.4 ± 2.0 | 30.1 ± 1.1§§§ |
| Mean score velocity[22] | 2.93 ± 0.01 | 1.57 ± 0.04 | 0.96 ± 0.05 | 0.97 ± 0.04 | 0.47 ± 0.04 | 0.61 ± 0.05 | 0.40 ± 0.05 | 2.25 ± 0.03 | 2.48 ± 0.02 | 2.38 ± 0.03 | 0.49 ± 0.04 | 2.51 ± 0.04 |
| Number of animals | 10 | 10 | 6 | 10 | 4 | 3 | 3 | 10 | 10 | 10 | 5 | 4 |
| Velocity score ratings | | | | | | | | | | | | |
| Total number of vessels | 715 | 885 | 408 | 711 | 323 | 245 | 252 | 946 | 863 | 673 | 218 | 463 |
| % Vessels, Score of 0 | 1 | 32 | 42 | 45 | 62 | 58 | 75 | 73 | 3 | 5 | 72 | 5 |
| % Vessels, Score of 1 | 1 | 12 | 26 | 22 | 29 | 24 | 11 | 111 | 5 | 7 | 14 | 11 |
| % Vessels, Score of 2 | 3 | 24 | 25 | 24 | 9 | 19 | 13 | 273 | 32 | 32 | 6 | 14 |
| % Vessels, Score of 3 | 95 | 32 | 6 | 9 | 0 | 0 | 1 | 489 | 59 | 55 | 7 | 71 |

BL: baseline; HBL1: hemorrhagic baseline 1; HBL2: hemorrhagic baseline 2; HBL: hemorrhagic baseline; Pre-IPR 5, 15, 30 min (Control): 1st control at min 5, 15 and 30, respectively; IPR 5 min, IPR 15 min, IPR 5 min: intrathoracic pressure regulation at min 5, 15 and 30, respectively; 2nd control at min 5: Post-IPR 5min (Control).
¶$p = 0.001$ for baseline vs HBL; ¶¶$p < 0.01$ for Baseline vs HBL; *$p = 0.002$ for IPR vs HBL; **$p = 0.005$ for IPR vs HBL; ***$p = 0.047$ for IPR vs HBL; †$p = 0.01$ for blood transfusion vs HBL; §$p = 0.006$ for IPR vs HBL; §§$p = 0.0001$ for IPR vs HBL; §§§$p = 0.04$ for blood transfusion vs HBL.

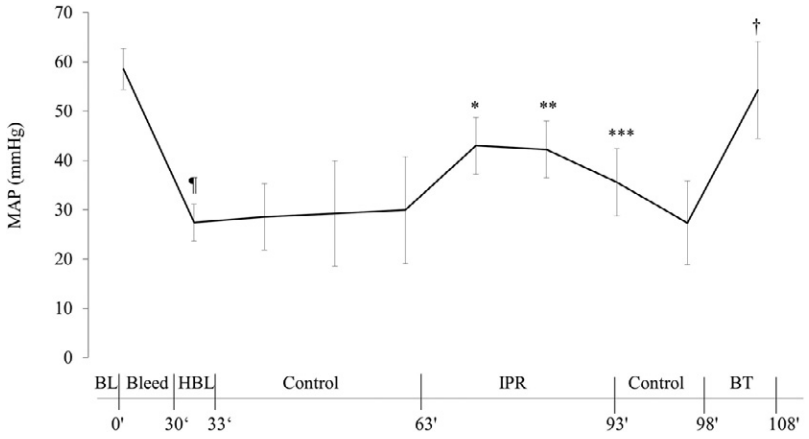


**Fig. 2.** Mean arterial pressure (mm Hg) ± SEM during various timepoints of the study. BL: baseline; HBL: hemorrhagic baseline; IPR: intrathoracic pressure regulation; BT: blood transfusion. ¶$p$ = 0.001: baseline > hemorrhage baseline; *$p$ = 0.002: IPR at 5 min > hemorrhage baseline; **$p$ = 0.005: IPR at 15 min > hemorrhage baseline; ***$p$ = 0.047: IPR at 30 min > hemorrhage baseline; †$p$ = 0.01: blood transfusion at 5 min > hemorrhage baseline

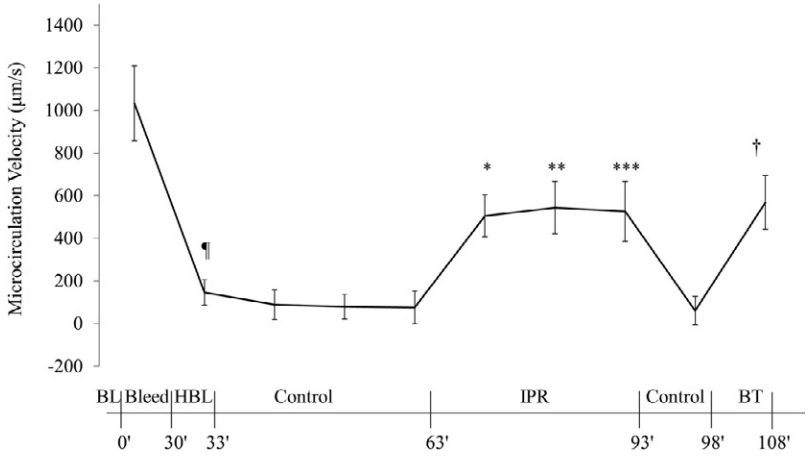


**Fig. 3.** Mean capillaries velocities (μm/s) ± SEM during various timepoints of the study. BL: baseline; HBL: hemorrhagic baseline; IPR: intrathoracic pressure regulation; BT: blood transfusion. ¶$p$ = 0.0001: baseline > hemorrhage baseline; *$p$ = 0.001: IPR at 5 min > hemorrhage baseline; **$p$ = 0.003: IPR at 15 min > hemorrhage baseline; ***$p$ = 0.008: IPR at 30 min > hemorrhage baseline; †$p$ = 0.002: blood transfusion at 5 min > hemorrhage baseline.

values remained nearly constant upon IPR application but the arterial pH values decreased, consistent with increased clearance of lactate concurrently with increases in macro and microcirculation. Upon removal of IPR, arterial pH and base excess decreased further. The partial pressure of $O_2$ remained >100 mm Hg throughout the study. Following the blood transfusion the arterial pH and base excess improved.

Microcirculation videos of the experiment are available online only.

## 4. Discussion

Results from this study demonstrate the relationship between systemic hemodynamics and microcirculation in an animal model of severe hemorrhage. There was a striking reduction in microcirculation when systemic blood pressure was severely reduced. When mean systolic blood pressures were reduced to 30 mm Hg, carotid blood flow velocity values decreased concurrently. Microcirculation, measured in terms of red cell velocity, came to a near standstill. By contrast to reduction in MAP from 80 mm Hg to 30 mm Hg, microcirculation blood cell velocity decreased from an average of 1033 ± 175 μm/s to 147 ± 60 μm/s. Both microcirculation and systemic hemodynamics were rapidly restored with IPR. These observations demonstrate the ability to non-invasively regulate intrathoracic pressure to improve tissue perfusion, by enhancing microcirculation and systemic pressures in the setting of severe hypovolemia. With IPR application MAP increased by approximately 60% to 80% of pre-hemorrhage values whereas microcirculation red cell velocity increased by approximately 344% to 50% of pre-hemorrhage values. In this study non-invasive therapy with IPR increased blood pressure and blood flow velocity to levels less than the baseline euvolemic levels. With blood transfusion, MAP levels were restored to baseline euvolemic levels but microcirculation values were approximately half of those measured at baseline and similar to those achieved with IPR.

MAP and microcirculation did not change to the same degree with hemorrhage until a critical MAP was achieved. A systolic blood pressure level of approximately 30 mm Hg appears to represent an on/off threshold for microcirculation in the setting of hemorrhagic hypotension in this animal model. In 6 animals microcirculation appeared grossly normal after a 50% hemorrhage and then plummeted when an additional 5% of the overall blood volume was removed as systolic pressures decreased to the target of 30 mm Hg. These observations suggest that microcirculation is protected in the setting of severe hypovolemia above a threshold driving pressure. Specifically, microcirculatory flow was severely reduced when systolic blood pressures decreased below 30 mm Hg, although there were some inter-individual differences in this threshold level. Another example of the discordance between MAP and microcirculation was observed in this study when the blood transfusion was delivered. Only 4 of 10 animals survived to the point that blood transfusion could be administered, given the severity of the bleed. With the

blood transfusion the hematocrit increased but not to a significant degree, likely due to the lack of statistical power with only 4 pigs having received the transfusion, the others dying prematurely. By contrast to the MAP, which increased to baseline pre-hemorrhage levels with the blood transfusion, the microcirculation increased to only half of the pre-hemorrhage values.

Prior studies with IPR demonstrate the negative intrathoracic pressure generated during each inspiration draws more blood back into the heart resulting in an immediate increase in stroke volume, cardiac output as well as systolic and diastolic BP in animal models of hypotension and in hypotensive patients.[16–19,25–28] The changes in intrathoracic pressures are also instantaneously transmitted to the brain presumably via the paravertebral veins surrounding the spinal column.[29] IPR lowers intracranial pressure and the resistance to brain flow results in an increase in cerebral blood flow velocity, and thus a reduction of symptoms of acute hypotension.[30] IPR has also shown to increase short term and 24 h survival in a porcine model of hypovolemic shock.[18,31] The current study demonstrated that application of IPR in the setting of severe hemorrhagic hypotension non-invasively reversed both microcirculation and systemic hemodynamic pressures without fluid resuscitation or blood transfusion. This is the first report to demonstrate that IPR increases microcirculation in the setting of severe hypotension. These findings are consistent with earlier reports demonstrating that IPR can provide non-invasive blood pressure support in animal models of blood loss and in patients.[22,23,31] The rise in aortic blood pressure by IPR reversed the decrease in microcirculation within a couple of min or less. These effects were accompanied by greater clearance of metabolites, as manifested by a decrease in arterial blood gas pH with IRP. This paradoxical worsening of the tissue metabolic profile with IPR is likely a direct result of the 344% rise in microcirculation with IPR application and the rapid clearance of acidosis at the cellular level.

One of the important methodological issues related to this study involves the method for quantitating mean arteriolar density. The mean arteriolar density was constant throughout the current experiment contrary to some other publications because we counted the vessels in which red blood cells were visible, regardless of whether or not there was blood flow.

There are several limitations of this study. First, microcirculation was limited to the sublingual mucosa and this may not be reflective of other parts of the body. This tissue is readily accessible and has been used by others as a reasonable site for microcirculation assessment.[2,3,9–11,24] Second, the animals were not subject to a splenectomy and that may have been why we observed a threshold value of 30 mm Hg before microcirculation plummeted. Third, acquisition of quality videographic recordings depends on the skill level of the device operator, and there is a substantial learning curve. The operator for these studies (NS) has significant prior experience with this technique.[32] To obtain quality images, some pressure must be applied to keep the device in position of the desired tissue area. If too much pressure is applied, however, the small and thin-walled capillaries can be compressed and lead to falsely decreased microcirculatory variables. Fourth, the mechanism of action of the IPR device was not elucidated. While it is presumed that the expiratory phase decrease in intrathoracic pressure augments forward flow by enhancing cardiac stroke volume and lowering resistance to forward brain flow, it is also possible that blood is “pulled through” rather than just “pushed through” the microvasculature capillary beds. Further research is needed in this regard.

## 5. Conclusion

Using SDM technology to measure sublingual microcirculatory flow during severe hypotension secondary to hemorrhage, application of a non-invasive device to regulate intrathoracic pressure during the expiratory phase of positive pressure ventilation resulted in a rapid rise in systemic arterial pressure and in red cell velocity through the microvasculature.

## Conflicts of interest

Doctors Lurie, Rees and Metzger have disclosed a relationship with Advanced Circulatory Systems. The other authors have disclosed no conflicts of interest.

The opinions and assertions in this paper are the private views of the authors and are not to be construed as reflecting the views of the United States Department of the Army or Department of Defense.

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